page 10, second last full paragraph to page 11, page 21, first full paragraph, page 27, second full paragraph, and originally filed claim 20. Additionally, these independent claims are related to claims 1-15 of Issued Patent No. 5,932,540.

Moreover, support for representative claims 34-35, can be found, for example, at page 18, first full paragraph and page 27, first full paragraph. Support for representative claims 36-39 can be found, for example, at page 23, second full paragraph and page 26, fourth full paragraph. Support for representative claims 40-41 can be found, for example, at page 26, fifth full paragraph. Additionally, support for representative claim 42 can be found, for example, at page 11, last full paragraph that extends to page 12. Support for representative claim 44 can be found, for example, at page 32, last full paragraph to page 33.

Furthermore, support for representative claims 44-53 can be found, for example, at pages 27, last full paragraph, to page 28, and are related to claims 61-185 of Issued Patent No. 5,932,540.

Thus, no new matter has been added by way of the amendment.

I. Amendment of the Specification.

The specification has been amended to correct an obvious typographical error. 5x SSC is a well-known solution used in hybridization solutions. (*See*, e.g., Exhibit A, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley and Sons, N.Y., at page 2.10.7 (1989).) SSC is normally made as a 20x stock solution, and then diluted accordingly for a particular use. Exhibit B shows that a 20x SSC stock solution contains 3 M NaCl and 0.3 M trisodium citrate. (*See*, e.g., Exhibit B, CURRENT PROTOCOLS, at page A.2.5.) To make a 5x SSC solution, the 20x solution must be diluted by a factor of four. Therefore, a 5x SSC solution contains 750 mM NaCl (3 M ÷ 4 = 750 mM) and 75 mM trisodium citrate (0.3 M ÷ 4 = 75 mM). One skilled in the art would have immediately recognized that the amount of ingredients listed in the specification for a 5x SSC solution was incorrect. Rather than describing a 5x SSC solution, made up of 750 mM NaCl and 75 mM trisodium citrate, the specification inaccurately listed the ingredient amounts for a 1x solution. The skilled artisan, in recognizing the typographical error, could have easily adjusted the amount of ingredients described in the specification to properly make a 5x SSC solution.

Therefore, because no new matter will be added to the specification if these typographical errors are corrected, Applicants respectfully request that the amendments to the specification to recite the correct ingredient amounts in 5x SSC be entered.

II. ATCC Deposit

To demonstrate full compliance with 37 C.F.R. §§ 1.803-1.809 and to satisfy the requirement of 35 U.S.C. § 112, first paragraph, Applicants assure the Examiner that ATCC Deposit No. 97149 has been deposited under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure with the following International Depository Authority: American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, USA. The deposit comprise a recombinant nucleic acid vector into which cDNA sequence encoding Vascular Endothelial Growth Factor 2 (VEGF-2) has been inserted. The deposit was made on May 12, 1995 as disclosed on page 8, lines 1-3 of the instant application.

In accordance with MPEP § 2410.01 and 37 C.F.R. § 1.808, assurance is hereby given that all restrictions on the availability to the public of the above nucleic acid molecule encoding human VEGF-2, will be irrevocably removed upon the grant of a patent based on the captioned application, and that the deposit will be replaced if viable samples cannot be dispensed by the ATCC, except as permitted under 37 C.F.R. § 1.808(b).

III. The Restriction Requirement.

The Examiner has required an election under 35 U.S.C. § 121 of one of Groups I-XII. In response, Applicants provisionally elect, *with traverse*, Group II represented by claims new claims 33-346 for further prosecution. Applicants reserve the right to file one or more divisional applications directed to non-elected inventions should the restriction requirement be made final.

Applicants respectfully request that the Examiner enter the following amendments prior to examination of the captioned application.

Applicants respectfully traverse the restriction requirement as it applies to Groups I and III-XII. As the Examiner points out, polynucleotides, polypeptides, antibodies, etc. are

patentably distinct inventions. However, even where two patentably distinct inventions appear in a single application, restriction remains improper unless it can be shown that the search and examination of both groups would entail a "serious burden". *See*, M.P.E.P. § 803.

In the present situation, no such showing has been made. Indeed, no arguments have been made explaining why it would impose an undue burden to examine Groups I-XII together.

Applicants submit that a search of the polypeptide claims would provide useful information for Groups I and III-XII. For example, in many if not most publications, where a published polypeptide sequence, the authors also routinely include a description of the polynucleotides and antibodies. Thus, the searches for polypeptides, polynucleotides, and antibodies, etc. commonly overlap. Thus, the search and examination of a polypeptide sequence, and remaining groups would not entail a serious burden. Thus, the searches for Groups I-XII would be overlapping.

Accordingly, as applied to Groups I-XII, the restriction requirement should be withdrawn.

Conclusion

In view of the foregoing remarks, applicants believe that this application is now in condition for allowance.

If there are any fees due in connection with the filing of this paper, please charge the fees to our Deposit Account No. 08-3425. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Dated: 12/15/99

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Enclosures
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CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

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John Wiley & Sons, Inc.

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IN SITU HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY

14

continued

1c. *Harsh treatment:* Pour several hundred milliliters of boiling 0.1% SDS onto the membrane. Cool to room temperature.

If a membrane is to be reprobed, it must not be allowed to dry out between hybridization and stripping. If it becomes dry, the probe may bind to the matrix.

2. Place membrane on a sheet of dry Whatman 3MM filter paper and blot excess liquid with a second sheet. Wrap the membrane in plastic wrap and set up an autoradiograph.

If signal is still seen after autoradiography, rewash using harsher conditions.

3. The membrane can now be rehybridized. Alternatively, it can be dried and stored for later use.

Membranes can be stored dry between Whatman 3MM paper for several months at room temperature. For long-term storage, place the membranes in a desiccator at room temperature or 4°C.

REAGENTS AND SOLUTIONS

Aqueous prehybridization/hybridization (APH) solution

5× SSC (*APPENDIX 2*)
5× Denhardt solution (*APPENDIX 2*)
1% (w/v) SDS
Add 100 µg/ml denatured salmon sperm DNA (see below) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

Denatured salmon sperm DNA

Dissolve 10 mg Sigma type III salmon sperm DNA (sodium salt) in 1 ml water. Pass vigorously through a 17-G needle 20 times to shear the DNA. Place in a boiling water bath for 10 min, then chill. Use immediately or store at –20°C in small aliquots. If stored, reheat to 100°C for 5 min and chill on ice immediately before using.

Formamide prehybridization/hybridization (FPH) solution

5× SSC (*APPENDIX 2*)
5× Denhardt solution (*APPENDIX 2*)
50% (w/v) formamide
1% (w/v) SDS
Add 100 µg/ml denatured salmon sperm DNA (see above) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

Commercial formamide is usually satisfactory for use. If the liquid has a yellow color, deionize as follows: add 5 g of mixed-bed ion-exchange resin [e.g., Bio-Rad AG 501-X8 or 501-X8(D) resins] per 100 ml formamide, stir at room temperature for 1 hr, and filter through Whatman no. 1 paper.

CAUTION: *Formamide is a teratogen. Handle with care.*

Labeling buffer

200 mM Tris·Cl, pH 7.5
30 mM $MgCl_2$
10 mM spermidine

Mild stripping solution

5 mM Tris·Cl, pH 8.0
2 mM EDTA
0.1× Denhardt solution (*APPENDIX 2*)

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IN SITU HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY

14

continued

SDS electrophoresis buffer, 5×

15.1 g Tris base
72.0 g glycine
5.0 g SDS
H_2O to 1000 ml
Dilute to 1× or 2× for working solution, as appropriate

Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted. Store at 0° to 4°C until use (up to 1 month).

SED (standard enzyme diluent)

20 mM Tris·Cl, pH 7.5
500 µg/ml bovine serum albumin (Pentax Fraction V)
10 mM 2-mercaptoethanol
Store up to 1 month at 4°C

Sodium acetate, 3 M

Dissolve 408 g sodium acetate·$3H_2O$ in 800 ml H_2O
Add H_2O to 1 liter
Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

Sodium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid/liter (0.2 M).
Solution B: 27.2 g sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 100 ml. (See Potassium acetate buffer recipe for further details.)

Sodium phosphate buffer, 0.1 M

Solution A: 27.6 g $NaH_2PO_4 \cdot H_2O$ per liter (0.2 M).
Solution B: 53.65 g $Na_2HPO_4 \cdot 7H_2O$ per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

SSC (sodium chloride/sodium citrate), 20×

3 M NaCl (175 g/liter)
0.3 M Na_3citrate·$2H_2O$ (88 g/liter)
Adjust pH to 7.0 with 1 M HCl

STE buffer

10 mM Tris·Cl, pH 7.5
10 mM NaCl
1 mM EDTA, pH 8.0

TAE (Tris/acetate/EDTA) electrophoresis buffer

50× stock solution:
242 g Tris base
57.1 ml glacial acetic acid
37.2 g $Na_2EDTA \cdot 2H_2O$
H_2O to 1 liter

Working solution, pH ~8.5:
40 mM Tris·acetate
2 mM $Na_2EDTA \cdot 2H_2O$

TBE (Tris/borate/EDTA) electrophoresis buffer

10× stock solution, 1 liter:
108 g Tris base (890 mM)
55 g boric acid (890 mM)
40 ml 0.5 M EDTA, pH 8.0 (20 mM)